REMARKS

The Office Action has been carefully reviewed. No claim is allowed. Claims 5, 9, 11, 12, 15-17 and 19 presently appear in this application and define patentable subject matter warranting their allowance. Reconsideration and allowance are hereby respectfully solicited.

Claims 5, 9, 11, 12, 15-17 and 19 have been rejected under 35 U.S.C. §112, first paragraph, for lack of enablement. This rejection is respectfully traversed.

The specific issues raised by the examiner in this rejection are addressed below.

(1) The pending claims are directed to a method for inhibiting angiogenesis in adipose tissue in mammals, by inducing angiopoietin-2, comprising administering to a subject a pharmaceutical composition comprising leptin, and optionally an inhibitor of angiogenesis. However, the examiner considers that in view of the state of the art and lack of support in the pending application, one skilled in the art would conclude that leptin would promote (rather than inhibit) angiogenesis, including in adipose tissue, of normal mammals, absent evidence to contrary.

Lack of support in the specification

The present specification teaches that leptin is effective for modulating angiogenesis and inhibiting unwanted

angiogenesis, especially angiogenesis-related tumor growth and adipose tissue growth (page 9, lines 15-17). This effect is mediated by angiopoietin-2, whose expression is induced by leptin in various tissues, including adipose tissues (page 9, lines 13-15).

The specification, including the examples, provides clear guidance on the way to carry out the presently claimed invention, along with different alternatives. Example 1 on pages 24-25 of the specification clearly demonstrates that the use of leptin on adipose tissues of adult C57BL *ob/ob* mice results in blood vessel regression (Fig. 1). As already mentioned in our reply dated February 19, 2008, the knock-out model of Example 1 is a well-accepted model for those of skill in the art. Just as the rat corneal model is a well-accepted model for studying angiogenesis, the knockout model is another well-accepted model for studying the effect of one given molecule/protein. Therefore, in view of the results obtained in Example 1, the **skilled person would know that leptin has an inhibitory effect on angiogenesis in adipose tissue**.

In addition, the examples clearly support this finding by demonstrating that the expression of Angiopoietin-2 (ang-2), which is a known angiostatic molecule in some conditions, is enhanced in adipose tissue under treatment with leptin. This effect has been demonstrated *in vivo*, on adipose tissue of C57BL

mice and C57BL *ob/ob* mice (Example 2), as well as *in vitro* on 3T3 F442A murine mature adipocytes (Example 3). One post-filing publication, by Cohen *et al.*, *J. Biol. Chem.* 276:7697-7700 (2001), submitted with our reply dated July 12, 2006, and of record, confirmed these results.

For the reasons above, and in view of the specification, applicants believe that one of skill in the art can indeed make and use the present invention without any undue experimentation.

Rat corneal model

Applicants agree that in view of Kenyon *et al.*, Park *et al.* and Hui-Chaun *et al.*, the rat corneal model appears to be a well-accepted model for studying the response to angiogenesis. However, it is not because this model is the only well-accepted model for angiogenesis that is available or even that it is the most appropriate model for the present application. This is evidenced by the Bouloumie *et al*, *Circulation Res.* 83:1059-1066 (1998), reference of record, whose authors did not perform their experiments using the rat corneal model. Other models are known and well-accepted by those of skill in the art, such as the knock-out model in mammals.

Leptin as an inducer of angiogenesis in normal mammals

Park *et al., Exp. Mol. Med.* 33:95-102 (2001), of record disclosed that **leptin has a role in angiogenesis** by inducing endothelial cell proliferation and expression of matrix metalloproteinases *in vivo* and *in vitro*. To investigate the effects of leptin on vascularization *in vitro* and *in vivo*, they performed experiments on **rat corneal cells, HUVECs (Human Umbilical Vein Endothelial Cells) and HCASMCs (Human Coronary Artery Smooth Muscle Cells)**. From this document, it appears that **leptin has a cell proliferative effect on HUVECs but not on HCASMCs** (Fig. 1A). Moreover, in view of Fig. 1A, a slight decrease of cell proliferation is observed when HCASMCs are treated with different concentrations of leptin.

Sierra-Honigmann *et al. Science* 28(5383):1683-1686 (1998),of record, showed that, *in vivo*, **leptin induced neovascularization in corneas from normal rats** but not in corneas from fa/fa zucker rats, which lack functional leptin receptors. *In vitro* angiogenesis assays were performed on **HUVECs**.

Bouloumie et al (1998), *supra*, taught that **leptin** stimulates endothelial cells, leading to an increase in cell proliferation and **promotes angiogenic processes**, on **HUVECs and PAECs (Porcine Aortic Endothelial Cells)**.

Cao et al, *PNAS* 98(11):6390-6395 (2001), of record, disclosed that leptin plays a critical role in the maintenance

and regulation of **vascular fenestrations in adipose tissue**. As previously argued in our reply dated August 1, 2008, such fenestration is not synonymous with capillary proliferation and obviously can occur following destruction of capillary walls.

In view of the above-cited references, it is clear that **none of the experiments**, carried out to study the effect of leptin on angiogenesis, **were performed on adipose tissues**, whereas the present claims are directed to a **method of inhibiting angiogenesis with leptin in adipose tissues**.

Moreover, in view of the results from Park et al, it appears that leptin has a different effect in different types of cells. This finding is also confirmed in Example 3 of the present specification, where leptin induces angiogenesis in mice pre-adipocytes and has angiostatic effect on mature adipocytes.

Thus, one of skill in the art, in view of the present specification and the state of the art, would certainly not conclude that leptin would promote angiogenesis in every kind of tissue/cell of mammals, including in adipose tissue. To the contrary, this same person **would instead conclude** that leptin has at least:

- an angiogenic effect on HUVECs, PAECs and rat corneal cells;
- no effect on HCASMCs; and

- an angiostatic effect on adipose tissue, more especially on mature adipocytes.

Therefore, the present claims are fully supported by the specification and meet the enablement requirements.

(2) The examiner also asserts that claim 19 is only enabled for ob/ob mice, absent evidence to contrary.

Holmes et al, *Genome Biology* 6:209-10 (2005), of record, teaches that VEGF-A expression declines in most tissues in the weeks after birth and is relatively low in most adult organs (page 209.6, right column, 1st paragraph). This protein is produced by diverse cell types such as aortic vascular smooth muscle cells or keratinocytes (page 209.6, right column, 1st paragraph). However, **adipose tissues were not described**.

Claim 19 is dependent from the method of claim 9, wherein, in the adipose tissue in which angiogenesis is inhibited, VEGF is absent. This claim is not directed only to adipose tissue of *ob/ob* mice but to adipose tissue in which VEGF is absent. This includes adipose tissue of *ob/ob* mice but it is certainly not limited to this specific mouse genotype, as supported by Example 3 of the present specification which shows that the expression of VEGF is lower in mature adipocytes. In order to assay the expression of VEGF in one given cell/tissue, one of skill in the art would certainly know the simple tests to be performed: at the protein level (ELISA assay) and/or at the

mRNA level (detection of VEGF mRNA with the help of labeled probes).

(3) The examiner takes the position that the presence or over-expression of a single gene (i.e., Ang-2) may not be the only factor that could control a complex process such as angiogenesis.

Teichert-Kuliszewska et al, *Cardiovascular Research* 49:659-670 (2001), of record, performed their experiments on **HUVECs, Rat aortic SMCs** (smooth Muscle Cells) and **human embryonic kidney**, grown on fibrin matrix. They taught that ang-2 can promote substantial capillary-like tube formation in an *in vitro* model of angiogenesis (page 665, left column, 2nd paragraph). In the meantime, Ang-2 may be an endogenous inhibitor of Ang1-induced Tie2 receptor activation (page 666, left column paragraph). Moreover, as disclosed in the abstract, the **role of Ang2 is more complex** than previously recognized, **acting alternately to promote or blunt Tie2 receptor signaling** in endothelial cells, **depending on local conditions**. It should be pointed out that **adipose tissues were not studied**.

The present specification clearly teaches that when C57BL *ob/ob* mice are treated with leptin, **both blood vessel regression** (Example 1) and **enhanced expression of ang-2** (Example 2) **are observed in adipose tissue**. Enhancement of Ang-2 expression is also obtained after treatment of 3T3 F442A

adipocytes with leptin (Example 3). Thus, Ang2 appears to act as an **inhibitor of angiogenesis in adipose tissues**, as its expression is correlated with blood vessel regression. Applicants agree with the fact that for such a complex process as angiogenesis, Ang-2 is probably not the only factor involved. However, applicants did not disclose that angiogenesis is due only to an enhancement of Ang-2 expression.

Therefore, in view of the Examples in the present specification, it is correct to view **leptin as acting as an inhibitor of angiogenesis in adipose tissue by inducing ang-2**, even if ang-2 is not the only factor involved.

Reconsideration and withdrawal of the rejection are therefore respectfully requested.

In view of the above, the claims comply with 35 U.S.C. §112 and define patentable subject matter warranting their allowance. Favorable consideration and early allowance are earnestly urged.

Respectfully submitted,

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